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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

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☐ *Additional inventors are being named on the _____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Compositions And Methods For Improved Respiratory Tract Mucus Clearance

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* 27 ☐ CD(s), Number

☒ Drawing(s) *Number of Sheets* 7 ☐ Other (specify)

☒ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____.

Respectfully submitted,

SIGNATURE _____ Date 03/30/04

TYPED or PRINTED NAME Anita Nador REGISTRATION NO. *(if appropriate)* 47,366

TELEPHONE 416-957-1684 Docket Number: 11157-76

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March 30, 2004

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Your Reference:
Our Reference: 11157-76

The Commissioner of Patents
& Trademarks
Washington, D.C. 20231
U.S.A.

Dear Commissioner:

Re: New US Provisional Patent Application
Title: Compositions And Methods For Improved Respiratory Tract Mucus Clearance.
Inventors: KING, Malcolm and ZAYAS, J. Gustavo

Enclosed herewith please find the following documents regarding the above New United States Provisional Patent Application that is being filed **March 30, 2004**.

1) Fee Transmittal
2) Provisional Patent Application Cover Sheet
3) Application Data Sheet.
4) Provisional Patent Application.

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UNITED STATES PROVISIONAL

Title: Compositions and Methods For Improved Respiratory Tract Mucus Clearance

Inventors: Malcolm King , J. Gustavo Zayas

Title: Compositions and Methods for Improved Respiratory Tract Mucus Clearance

Field of the Invention

This invention relates to compositions and methods for improved respiratory tract mucus clearance

Background of the Invention

The Severe Acute Respiratory Syndrome (SARS) has been shown to be caused by the SARS virus, a coronavirus never before seen in humans. Since November 2002, when the first case of this new disease was detected, several thousand patients have been reported in more than 20 countries, with more than 300 cases and 23 deaths across Canada. How SARS is transmitted remains to be clearly defined; however the group of researchers who identified the SARS virus consider that close person-to-person contact is the major way the disease spreads. They also acknowledge that diseases spread by droplets are the most difficult to control.

Since SARS is believed to be an airborne disease, it is critical to focus attention on the respiratory system. Two main complementary defense mechanisms protect the airways. Mucociliary clearance is the first line of defense and cough clearance is the backup or reinforcement if the first one is unable to carry out the task. Mucus plays a critical role in both clearance mechanisms (King M, Rubin BK. Rheology of airway mucus: Relationship with clearance function. Chapter 7 of: Takishima T, Shimura S, eds. *Airway Secretion: Physiological Bases for the Control of Mucus Hypersecretion (Lung Biology in Health and Disease Series)* New York: Marcel Dekker, 1994, 283-314).

Airborne diseases are transmitted when an infected person coughs, sneezes and perhaps even speaks.

When the airway mucus layer interacts with high-speed airflow as in coughing, there is formation of droplets of different sizes that are expelled to the surrounding environment as an aerosol. The concentration of droplets and their size distribution each play an important role in transmission of

airborne diseases or infections, such as SARS and other diseases such as influenza and tuberculosis.

Airway mucus derives from the goblet cells of the epithelial surface layer and the mucous cells of the submucosal glands. The mucous secretion is a non-homogeneous, viscoelastic fluid containing glycoproteins, proteins, and lipids in a watery matrix. The mucus along with serous fluid forms the airway surface fluid (ASF) that provides a protective milieu for the airways. The composition and physical characteristics of ASF allow for normal ciliary activity and airway protection (6). When disruption of normal secretory or mucociliary clearance processes occurs, respiratory secretions can accumulate and impair pulmonary function, reduce lung defenses and increase the risk for infection and possibly neoplasia (14-16).

Mucomodulator therapy – changing the physical properties of ASF – is designed to enhance the clearance of mucus from the respiratory tract as well as to optimize aspects of lung defense that depend on the mucous layer. Mucomodulators include mucolytics, designed to disrupt the structural macromolecules that give respiratory tract mucus its physical characteristics, and other agents designed to increase mucus flow by stimulating ciliary activity or improving periciliary fluid hydration. Mucomodulator therapy combating mucus retention is a major consideration in the treatment of cystic fibrosis and other chronic lung diseases in which mucus hypersecretion and impaired airway clearance produce symptoms (17,18).

Mucous factors affecting MCR are the mucus depth and mucus viscoelastic properties (6,12). When the mucus layer is too thick and clearance by the cilia is hindered, clearance by coughing takes over. Mucus needs to be both viscous and elastic. The elasticity of mucus is important for clearance by cilia because it efficiently transmits energy without energy loss. The viscosity of mucus results in energy loss, but it is necessary so that mucus can be displaced and either expectorated or swallowed. A balance between these factors must be maintained for optimal MCR.

Mucus that is elastic may be efficient in mucociliary clearance, but it is inefficient in cough clearance (13), and thus a dynamic balance between mucus viscosity and elasticity may be determined by nature. The effects of

mucolytic treatments on both forms of clearance should be considered in evaluating their efficacy.

There is a need to for a pharmacological intervention aiming at modulating the physical and biochemical characteristics of the respiratory secretions in order to minimize aerolization or expectorated material carrying the Infection. There is also a need to minimize the effect on mucociliary clearance. There is also a need to improve mucus clearance, such as mucociliary clearance and cough clearance (expectoration).

Summary of the Invention

The transmission of an airborne disease requires a transmissor (an individual with the disease) and a recipient (a healthy individual). Knowledge of the dynamic that takes place among the transmissor (e.g. a SARS patient), the recipient (healthy individual) and the surrounding microenvironment between them, the aerosolization that is required for transmission of airborne diseases, will provide information about the mode of transmission. Mucomodulation will reduce or prevent the spread of airborne diseases from the transmissor, as well as enhance the protective function of the mucus barrier in the recipient, when administered to these respective individuals.

As such, in one aspect, the object of the invention is to administer a mucus thickening agent to a patient to inhibit, prevent and/or treat a medical condition. In another aspect, the object is to inhibit transmission of a medical condition.

In one embodiment, the invention provides a method of inhibiting aerosolization of an airborne disease comprising administering an effective amount of a mucothickening agent, preferably a respiratory tract mucothickening agent to a patient in need thereof. In another embodiment, the method is to inhibit transmission of airborne disease. In yet another embodiment, the invention provides a method of treating a disease related to thin mucus or cilia malfunction or non-function, comprising administering to a patient in need thereof a mucothickening agent.

In one embodiment, the mucothickening agent is selected from the group consisting of those that promote the formation of one or more of the

following: covalent bonds, ionic bonds, hydrogen bonds, van der Waals' forces, intermingling, extracellular DNA & F-actin network. In another embodiment, the mucothickening agent is selected from the group consisting of: high molecular weigh dextran, sodium tetraborate, calcium chloride (or other source of divalent cations), and a polycationic agent. In another embodiment, the mucothickening agent is one or more of the said agents. In a further embodiment the mucothickening agent is a polycationic agent such as polylysine.

In another embodiment, the medical condition is an airborne disease. In another embodiment, the airborne disease is selected from, but not limited to, the group consisting of SARS, tuberculosis, and influenza.

In another aspect, the invention provides a method for thickening mucus, preferably respiratory tract mucus, comprising administering an effective amount of a polycationic agent, such as polylysine.

In another embodiment, the invention provides a method of diagnosing a patient with a medical condition comprising administering an effective amount of mucothickening agent to said patient and screening said mucus for said medical condition.

In another embodiment, the inventor provides compositions comprising a mucothickening agent suitable for use in the methods of the present invention, as described herein.

In another embodiment the invention provides a method of inhibiting or decreasing aerosolizable respiratory secretions in a way to minimize the effect on mucus clearance. Mucus clearance can be mucociliary clearance and/or cough clearance (expectoration). In one embodiment, the method of the invention can be achieved by administering to a subject an effective amount of a mucomodulator, such as a mucothickening agent.

In one embodiment, the invention provides a method of improving mucus clearance, such as mucociliary and/pr cough clearance by administering to a subject an effective amount of a mucomodulator, such as a mucothickening agent. In one embodiment, the mucothickening agent is sodium tetraborate or high molecular weight dextran.

In another embodiment, the invention provides a method of inhibiting aerolization of an airborne agent from the respiratory tract while minimizing

the effect or enhancing mucus clearance, such as mucociliary clearance and/or cough clearance.

Other features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

Brief Description of the Drawings

The invention will now be described in relation to the drawings in which:

Figure 1 is a diagram of the various types of bonds occurring in respiratory tract mucus

Figure 2 are bar graphs illustrating the effect of dextran molecular weight on the visoelasticity (A) and spinnability (B) of respiratory tract mucus of cystic fibrosis patients.

Figure 3 are scanning electron micrographs illustrating the effect of $CaCl_2$ on the mucus from the frog palate. Figure 2(A) is the mucus treated with Frog Ringers alone and Figure 2 is with $CaCl_2$.

Figure 4 is a bar graph illustrating the effect of the mucomodulator $CaCl_2$ on mucociliary clearance in the frog.

Figure 5 are bar graphs illustrating the effect of cross-linking agent (sodium teraborate) on mucocillarly clearance (5A) and cough clearance (5B)

Figure 6 are target bullseye results illustrating the effect of cross-linking agent (sodium tetraborate) on the aerosolization and dispersion of synthetic mucus in a cough simulator.

Figure 7 is a graph illustrating the effect of saline, sodium tetraborate (XLB) and high molecular weight dextran (XL D) on tacheal mucociliary clearance in anesthetized dogs.

Detailed Description of the Invention

"Inhibiting aerosolization" as used herein means reducing the amount of aerosolization as compared to a contol or as compared to the degree of aerosolization in a subject that was not administered an effective amount of mucomodulator , such as a mucothickening agent, in accordance with the method of the invention.

'Minimizing the effect on mucus clearance" as used herein means reducing the degree of difference in mucus clearance in a subject receiving the treatment of the present invention (administration of a mucomoudlator, such as a mucothickening agent) versus a contol or one that does not receive such treatment or some other selected baseline.

"Enhancing" or "improving" mucus clearance, as used herein means increasing mucus clearance as compared to a control or a subject that does not receive the treatment of the present invention or some other determined baseline.

"Mucomodulation" as used herein means modifying the rheological (viscoelasticity) properties of mucus by altering the viscosity and/or elasticity.

"Mucomodulator" as used herein means an agent designed or intended to produce mucomodulation of mucus.

"Mucothickening agent" as used herein means a mucomodulator designed or intended to produce a thicker mucus

"Thick" as used herein means viscous and/or elastic and/or rigid, in reference to the physical properties of mucus. In more scientific terms, "thick" means of high modulus of viscoelasticity (the capacity to deform and flow under applied pressure. "Thin" will have a converse meaning.

Thick mucus, such as seen in adult patients with cystic fibrosis, would have an elastic modulus in the range of 195-1780 dyn/cm2 and a viscosity in the range of 7.4-70 Poise, when measured at a frequency of 10 radians per second in a magnetic rheometer (Tomkiewicz R et al., Am Rev Respir Dis 1993; 148: 1002). Normal mucus, as sampled from the trachea of healthy

volunteers by bronchoscopy, exhibits an elastic modulus in the range 107-490 dyn/cm2 and a viscosity in the range 3.8-17.4 Poise (Jeanneret-Grosjean et al., Am Rev Respir Dis 1988; 137: 707-710). Thin mucus has lower elastic modulus and viscosity than normal. See appended sputum reference data in TABLE 1.

"Respiratory Tract Mucus" as used herein means mucus lining the respiratory epithelium, including the nasal passages and the tracheobronchial airways.

"High molecular weight dextran" as used herein means mean molecular weight ca. 500,000 Daltons or greater, more preferably in the range of 500,000 – 2,000,000, as assayed by conventional viscometric techniques.

The present invention provides a novel mucotherapy intended to modulate the physical characteristics of viscoelasticity, cohesivity and surface tension of the respiratory secretions to minimize the aerosolization that is required for transmission of airborne diseases. The invention does the opposite of what mucolytic agents traditionally do. In this aspect, the invention provides methods and compositions to reduce the aerosolizability of respiratory secretions while maintaining mucociliary clearability, and thus normal airway clearance function. This requires more subtle manipulation of mucus viscous and elastic properties than conventional mucolytic therapies offer (King & Rubin 2002). Ninety-five per cent of mucus is water and the remaining 5 % is made up of proteins, carbohydrates, salts, etc. Therefore, when the airway mucus layer interacts with high-speed airflow during coughing, there is formation of droplets of different sizes that are expelled to the surrounding environment as aerosol. The concentration of droplets and their size distribution may each play an important role in transmission of SARS when an infected person coughs or sneezes, and even possibly when speaking.

The number of infective agents (species) released to the environment are related to the amount expectorated, and the droplet size will determine their fate. Micron-sized droplets dry quickly and can remain airborne for long periods and possibly reach many persons through the environment via a common system: ventilation, water, heating. In contrast, larger droplets are propelled onto the nearest surface: respiratory, orogastrointestinal or ocular

mucosas of a nearby person, or settle readily to the floor. Regarding transmission larger particles may have an immediate effect - direct but limited action - while micron-size particles may have more indirect effects with longer-lasting and widespread consequences. The present invention addresses the question of which is the proper balance in modulating the size of the droplets.

The therapy works by increasing crosslinking binding sites in the mucin glycoprotein gel network, thereby raising mucin gel viscoelasticity and/or forming poorly soluble mucin complexes. The result is a less aerosolizable respiratory secretion, which could decrease the degree of contagiousness.

The mucus macromolecule consists of a protein core surrounded by short oligosaccharide side-chains, held together by different links: O-glycosidic bonds, disulphide bridges, hydrogen bonds and ionic bonds. These links are the targets of the existing mucolytic agents (King M, Rubin BK. Pharmacological approaches to discovery and development of new mucolytic agents. Advanced Drug Delivery Reviews 2002; 54: 1475-1490 – Figure 1).

The bonds that keep mucus together and effect viscoelasticity are depicted in Figure 1 and include: covalent bonds, ionic bonds, van der Waals' forces, intermingling, and extracellular DNA & F-Actin

Breaking covalent bonds reduces mucin molecular weight and results in changes to both mucociliary and cough clearability, as predicted by viscoelasticity measurements. Further, the reverse mucolyis needed in the present study requires the use of nontoxic treatments. For example, glutaraldehyde will increase mucus crosslink density, but this would be toxic. Disrupting ionic and/or hydrogen bonds produces more subtle effects on viscoelasticity, since only side-chain interactions are affected. The approaches to be tested include increasing ionic interactions by adding divalent cations, increasing H-bond crosslinking with agents like high molecular weight dextran or other high MW polysaccharides, and increasing specific interaction between side chain sugars, as with sodium tetraborate or other tetrafunctional anions which will selectively crosslink galactose units.

As such, a number of different mucothickening agents can be used in the invention that target the same or different bonds, noted above. These

could be applied alone or in combination, in order to minimize the change to mucociliary clearability, while reducing aerosolizability of the secretions.

One approach is to increase the concentration of divalent cations in the mucus through the administration of calcium and/or magnesium solutions. Conceptually, this is the opposite of what occurs in nature during mucin exocytosis, where intracellular mucin granules which were held tightly through calcium ion crosslinks give way to much looser interactions as sodium ions exchange for calcium during fusion with the apical membrane. An example of the use of this method is shown in the experiments illustrated in Figure 3. In this example, using the ex-vivo frog palate model, an increase in mucus clumping with calcium ion administration while maintaining mucociliary clearance was observed.

A second approach is to administer high molecular weight dextran (*ca.* 500,000 m.w.). The approach to using low molecular weight dextran as a mucolytic agent has been developed by Dr. King and collaborators (Feng *et al.*, 1998). This therapy has passed a phase 1 trial, and is currently in a phase 2 clinical trial in patients with cystic fibrosis. Low molecular weight dextran serves to reduce mucin gel crosslinking by disrupting intermolecular mucin-mucin H-bond crosslinks, substituting instead mucin-dextran crosslinks which are dysfunctional for network formation. On the other hand, HMW dextran has approximately the same molecular weight as the subunits of mucin macromolecules; in this case mucin-dextran crosslinks are approximately as effective as the original mucin-mucin crosslinks (Figure 2). Interestingly, HMW dextran tends to raise elasticity relative to viscosity (as indicated by the increase in spinnability relative to log G*), thus its use would tend to inhibit aerosolizability, which will depend on spinnability, while maintaining mucociliary clearability. Suprisingly, it has been found to also improve or increase respiratory tract mucus cough clearacnce.

A third approach is to use sodium tetraborate solutions, which cause reversible crosslink formation between galactose units, which are the major neutral sugar component of mucins. In model studies using vegetable polysaccharides, sodium tetraborate preferentially raises elasticity relative to viscosity, and would favour mucociliary clearability at the expense of cough clearability and aerosolizability (King & Rubin 1994).

Fourth, polycationic agents, such as polylysine or polyarginine can be used as mucothickening agents.

There are potential benefits for the patients, for the caregiver and also for the general population. The mucomodulators of the present invention would represent containment for the outbreak and would allow more time to make studies and adjustments in the strategies designed to reduce casualties. The route of administration is preferably by inhaled aerosol dispensed by affordable mask inhaler or other types of aerosol administration devices known in the art.

The transmission of SARS and similarly transmitted diseases can be controlled by implementing a pharmacological intervention aiming at modulating the physical and biochemical characteristics of the respiratory secretions in order to minimize expectorated material that carries the infection.

Applications

The method and compositions of the invention can be used to modulate the viscoelasticity of respiratory tract mucus. It can be used to thicken said respiratory secretions to minimize the aerosolization of virus or bacteria-containing secretions during cough or sneezing. It can also be used to minimize the effect on mucociliary clearance or improve mucus clearance, such as mucociliary clearance and/or cough clearance. It can also be used to thicken said respiratory secretions to enhance the protective effect of the respiratory mucus layer against uptake of airborne pathogens in the recipient. As such the methods and compositions of the invention can be used to treat conditions where the mucus is too thin or where cilia is non-functioning. It can also be applied to thicken the mucus layer in other parts of the body where additional protection against pathogens would reduce the risk of infection. (e.g. respiratory or non-repiratory tract mucus). Such diseases include but are not limited to bronchiectasis and some types of chronic bronchitis by administering a therapeutically effective amount of said mucothickening agent to a patient in need thereof.

In another embodiment, the methods and compositions of the invention can be used to reduce, preferably inhibit, more preferably prevent, the

transmission of airborne transmissible conditions, such as, but not limited to SARS, influenza, and tuberculosis. The invention works by entrapping said viral or bacterial particles in a mucus that permits the mucus to be expectorated from the lungs (e.g. 10 microns or greater, above the normal respirable range), but minimizes formation of small viral or bacterial containing particles (e.g. 2 microns or less), that can aerosolize upon coughing and remain suspended for a period of time, and thus be spread more widely.

The invention can also be used to obtain a mucus sample and analyze its contents for diagnosis of medical conditions e.g. by screening of viral and bacterial particles or cells in said mucus using screening methods known in the art, to determine whether the patient has a mucoviscoelastic-related condition (too thick or too thin), to determine appropriate dosage range of the mucomodulator, preferably muco-thickening agent. Said dosage range can be determined by taking a mucus sample, subjecting it to various dosages of mucomodulator, and then by assessing the effect of said mucomodulator on the viscosity of said mucus. Determining the suitable dosage of said mucomodulator to get the desired mucomodulatory effect. Preferably said mucomodulator is a mucothickening agent and the effect is to thicken the mucus.

Pharmaceutical Compositions

The above described mucomodulators may be formulated into pharmaceutical compositions for administration to subjects in a biologically compatible form suitable for administration *in vivo*. By "biologically compatible form suitable for administration *in vivo*" is meant a form of the substance to be administered in which any toxic effects are outweighed by the therapeutic effects. The substances may be administered to living organisms including humans, and animals.

Administration of a therapeutically effective amount of pharmaceutical compositions of the present invention is defined as an amount effective, at dosages and for periods of time necessary to achieve the desired result. For example, a therapeutically active amount of a substance may vary according to factors such as the disease state, age, sex, and weight of the individual,

and the ability of the substance to elicit a desired response in the individual. Dosage regimes may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

An active substance may be administered in a convenient manner such as by direct application to the respiratory tract mucus, e.g. by injection (subcutaneous, intravenous, etc.), but most preferably by inhalation, such as through an inhaler or respiratory mask. Depending on the route of administration, the active substance may be coated in a material to protect the compound from the action of enzymes, acids and other natural conditions that may inactivate the compound. It may be delivered as a nebulized solution or suspension in an appropriate vehicle or as a dry powder formulation, using an appropriate breath-acuated device.

The compositions described herein can be prepared by *per se* known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985) or Handbook of Pharmaceutical Additives (compiled by Michael and Irene Ash, Gower Publishing Limited, Aldershot, England (1995)). On this basis, the compositions include, albeit not exclusively, solutions of the substances in association with one or more pharmaceutically acceptable vehicles or diluents, and may be contained in buffered solutions with a suitable pH and/or be iso-osmotic with physiological fluids. In this regard, reference can be made to U.S. Patent No. 5,843,456. As will also be appreciated by those skilled, administration of substances described herein may be by an inactive viral carrier.

With regard to administration by inhalation, suitable preparations can be prepared in accordance with methods known in the art. (See for example: Finlay WH, Lange CF, King M, Speert DP. Lung delivery of aerosolized dextran. Am J Respir Crit Care Med 2000; 161: 91-97; Hardy JG, Everard M, Coffiner M, Fossion J. Lung deposition of a Nacystelyn metered dose inhaler

formulation. J Aerosol Medicine 1993; 6:37-44; Vanderbist F, Wery B, Baran D, Van Gansbeke B, Schoutens A, Moes AJ. Deposition of nacystelyn from a dry powder inhaler in healthy volunteers and cystic fibrosis patients. Drug Dev Ind Pharm 2001; 27:205-12).

In one embodiment a 10-100nmol solution of $CaCl_2$ is administered to effect "clumping" while still maintaining mucociliary clearance. In another embodiment, sodium tetraborate is administered at a 1-10nmol concentration to an effect the addition of crosslinks to galactose-containing macromolecules. In yet another embodiment, high molecular weight dextran is administered at about the 4% concentration range, while poly-L-lysine can increase mucus crosslinking at a concentration of less than 1%. In one preferred embodiment, the neutral polysaccharides and cationic polymers are administered by means of dry powder inhalers, and thus the target concentration will be the pure material, i.e. 100%. In another embodiment, calcium and borate solutions are delivered by conventional wet nebulizers.

(h) Kits

The reagents suitable for carrying out the methods of the invention may be packaged into convenient kits providing the necessary materials, packaged into suitable containers. Such kits may include all the reagents required to perform the method of the invention, e.g., the mucomodulator, and optionally the inhaler or other physical device used for administration of the mucomodulator, and optionally a set of instructions for using the kit and components thereof in the methods of the invention. The kit may also include muco-collective devices to collect mucus samples for carrying out diagnostic test and assessments of the invention.

The following non-limiting examples are illustrative of the present invention.

EXAMPLES

Methods

Mucus Viscoelasticity:

Mucus can be considered a viscoelastic fluid, since it exhibits both liquid-like (viscous) and solid-like (elastic) properties (1). *Viscosity* is the

resistance to flow and represents the capacity of a material to absorb energy as it moves. *Elasticity* is the capacity of a material to store the energy used to move or deform it. The relative proportions of elasticity and viscosity are as important in describing how a material such as mucus behaves when it is subjected to external forces, as are the absolute values of either parameter by itself.

The magnetic microrheometer technique is used to measure the viscosity and elasticity of mucus (King M. Magnetic microrheometer. In: Braga PC, Allegra L, eds. *Methods in Bronchial Mucology.* New York: Raven Press, 1988, 73-83). A 100 μm steel ball is positioned in a 5-10 mg sample of mucus, and the motion of this sphere under the influence of an electromagnet is used to determine the rheological properties of the mucus. The image of the steel ball is projected via a microscope onto a pair of photocells, whose output is amplified and transmitted to a digital storage oscilloscope. By plotting the displacement of the ball against the magnetic driving force, the viscoelastic properties of the mucus can be ascertained. Two derivative parameters - mucociliary clearability index (MCI) and cough clearability index (CCI) - are computed from *in vitro* relationships (King M. Role of mucus viscoelasticity in cough clearance. Biorheology 1987; 24: 589-597).

Cough Clearability Assay:

The simulated cough machine system comprises the following elements: A 10-L tank with compressed air which serves as a pressure reservoir that generates airflow, simulating the lungs during a cough maneuver. The model trachea is a 1-m long rigid acrylic tube long with a rectangular 1x2 cm cross-section. Gas release from the tank is controlled by a solenoid valve located between the pressure reservoir and the model trachea. An aliquot of mucus or sputum is layered on the bottom of the model trachea and driven forward by pressurized gas to simulate the airflow pattern of a human cough (King M, Brock G, Lundell C. Clearance of mucus by simulated cough. J Appl Physiol 1985; 58: 1776-1782). The distance traveled by mucus samples under standardized cough-simulating airflow is used as a measure of cough clearability.

The depth of mucus and the airflow linear velocity are critical determinants of cough clearance. Mucus physical properties that are important to cough clearance are the viscosity of the mucus, and the elastic component. This latter component impedes forward motion and results in recoil after the cough event. Cohesivity allows mucus to hold together, and contributes to the surface properties, both on the air-mucus interface, as well as at the interface with the pericilliary layer. Mucus that is elastic may be efficient in mucociliary clearance, but inefficient in cough clearance. The dynamic balance between mucus viscosity and elasticity may be altered in airway pathologies associated with mucus.

Frog Palate Preparation:

From a bullfrog, *Rana catesbiana*, the upper portion of the head is removed modifying the procedures described in previous works. This is done by pithing the frog after lowering the body temperature to abolish pain sensation, and then cutting with scissors through from the junction of the posterior pharynx and esophagus out to the skin of the back. The palate is checked for macroscopic lesions, such as ulcers, or evidence of inflammation. The palate is then placed inside the frog chamber, a wooden box with a glass top and fitted glass front and manipulated through glove openings, and viewed under a dissecting stereomicroscope provided with a reticulated eyepiece. Humidity inside the box is maintained at 100% using a Pari nebulizer; the box is maintained at room temperature (22° to 24° C). Before carrying out any measurement, the palate is allowed to stabilize inside the box for 15 minutes.

Mucus Transport Velocity (MTV):

Mucociliary clearance is determined by observing the movement of particles of charcoal powder gently deposited on a sample of mucus on the palate surface; its clearance is visually monitored and hence MTV determined. The displacement of a 1 – 5 µL of endogenous frog mucus sample is timed as the trailing edge moves across a predetermined segment. MTV is calculated by dividing the distance traveled by the time elapsed, based on at least five measurements of the time required for the mucus sample to travel the defined distance. (Rubin BK, Ramirez O, King M. Mucus-depleted frog palate as a

model for the study of mucociliary clearance. J Appl Physiol 1990; 69: 424-429).

Cilia Beat Frequency:

Studies on cilia beat frequency *in situ* on the excised fresh frog palate are carried out to assess the earliest effects of the agent being tested (King M, Festa E. The evolution of the frog palate model for mucociliary clearance. In: Baum G, ed. *Cilia, Mucus and Mucociliary Interactions*. New York: Marcel Dekker, 1998, 191-201). These studies involve the use of a microscope acquisition system capable of acquiring digital video images of beating cilia on the surface of the excised frog palate. The action of the cilia can be correlated with other parameters of mucus transport for an enhanced understanding of the role of the cilia in mucociliary transport in health and disease.

Scanning Electron Microscopy:

Samples of mucus and tissue are placed in 2.5 % glutaraldehyde solution immediately after collection and stored at 4°C until processing. Briefly, the samples are post-fixed in 1 % osmium tetra oxide in Millonig's buffer at room temperature for one hour. They are then washed briefly in a series of ethanol (50 – 100 %), ten minutes at each step, followed by two additional periods of absolute ethanol (10 minutes each). The samples are further dehydrated by critical point drying at 31°C for 5 – 10 minutes, then mounted on a specimen holder for SEM and dried overnight in vacuum desiccators. In the final stage of preparation for viewing, the samples are sputter coated with gold (Edwards, model S150B Sputter Coater). Samples are viewed using SEM (Hitachi S-2500). Images are scanned directly to a computer and stored as image files for subsequent viewing. Ultramicroscopy studies help to assess the clumping effect of the drugs under test, as well as to evaluate if there is cellular exfoliation as a result of mucomodulators use.

Example 1 – Effect of dextran at various molecular weights on mucus

Low molecular weight dextran is a potential mucolytic treatment for cystic fibrosis lung disease currently in Phase 2 trials. In pre-clinical testing, it

promoted clearance by both mucociliary and airflow mechanisms, and the drug has passed a Phase 1 safety trial in normal subjects. High molecular weight dextran, as evidenced from the graphs below would not be suitable as a mucolytic agent, but shows interesting effects in terms of predicted mucociliary and cough clearance. A reduction in log G* at 1 rad/s (upper graph) is a primary predictor of improved mucociliary clearability (King & Rubin 1994) (Figure 2A), while a reduction in spinnability (Figure 2B) predicts improved cough clearability. The data suggest that high molecular weight dextrans, particularly if combined with other proposed treatments, might differentially affect cough and mucociliary clearability to produce the desired combination of reduced cough clearability while maintaining mucociliary clearance function.

Example 2 – Effect of $CaCl_2$ on mucus

Figure 3A is a scanning electron micrograph of mucus from the frog palate which had been treated with Frog Ringers solution. In mucus clearance studies on the frog palate, Frog Ringers is used as a control solution and mucus clearance or velocity is expressed relative to Frog Ringers. Mucus is mostly composed of water (95%), the remaining 5% is made up of glycoproteins linked together by various types of chemical bonds. During a cough or sneeze, air forced through the airways picks up minute mucus droplets which are expelled from the body as aerosol. The dispersion of the aerosol mist depends on the droplet size.

In the SE Micrograph, Figure 3B, the effect of $CaCl_2$ (100mmol solution), applied topically to the frog palate is shown. $CaCl_2$ causes "clumping" of the mucus on the surface of the palate. The ciliated surface of the palate can be seen below the mucus and does not appear different to control views (Figure 3A). The effect of the mucomodulating agent is to cause the mucus to form into "clumps" on the palate surface. This action suggests the aerosolizability of the mucus or the ability to form tiny droplets may be decreased. This action may have a positive benefit relative to the dispersion of a mucus mist during a cough or sneeze.

Example 3 – The effect of a mucomodulating agent ($CaCl_2$) on mucociliary transport.

In Figure 4, the effect of a mucomodulating agent on mucociliary transport time is shown compared to Frog Ringers solution. Both solutions (FR and $CaCl_2$) were applied topically to the palate in a volume of 4 µl. Two minutes was allowed for dispersion of the agent on the palate, followed by the measurement of mucociliary transport time. A droplet of mucus from the palate was placed on palate toward the mouth opening. Ciliary action transported the droplet down the palate. The measurement process (timing the movement of the droplet down the palate over a set distance of 4 mm as observed through a steriomicroscope with a reticulated eyepiece) was repeated five times and averaged to give a mean transport time of ± SD for each solution. It can be seen from the graph above, that no effect on mucocillary transport time was observed at either concentration of mucomodulating agent compared to Frog Ringers.

Example 4 – Animal and *in vitro* effect of mucomodulators on mucus clearance and disease transmitability

The effect of mucotherapy *in vitro*, in animals and in humans can be studied. The novel mucotherapy is designed to minimize respiratory aerosol emissions while interfering only minimally with normal mucus clearance mechanisms. In the mucus samples rheological, physiological and biochemical parameters, in addition to ultrastructural parameters as visualized through scanning electron microscopy can be studied using techniques described herein in combination with those known in the art. For instance, patients having potential SARS or SARS-like symptoms can be incorporated in an open clinical trial with historical controls.

To determine acute effects the inventors environmental exposure ex-vivo animal model can be used as described above. To measure mucociliary transportability we a modified frog palate technique can be used as described above. The method of the invention can also be used to identify the effects of mucomodulators on the aerosol pattern while coughing, sneezing and speaking with or without treatment of mucomodulators as well as to estimate

optimum dose and delivery system to reduce aerosol formation without compromising airway mucus clearance.

Aerosol drug delivery optimization, using bench tests and mathematical deposition models, can be used.

Mucus viscoelasticity can be determined by magnetic microrheometry, and anatomical epithelial injury by SEM. Real time ciliary beat frequency can be assessed in an optical microscope system capable of acquiring digital video sequences. Studies of aerosol pattern can be carried out in a controlled environment.

Dosing of mucomodulators

Assessment of the use of mucomodulators under varied conditions will be studied. Therefore, studies must provide within-study flexibility in dosage. Inhaling the mucomodulator may result in a wide intersubject variation in the effective dose due to differences in inhalation techniques. Aerosol drug delivery optimization, using bench tests and mathematical deposition models, will allow determination of a recommended technique and reduce this problem. The introduction of subjects to mucomodulators can be conducted using low initial doses of the component with increases in dose being made only after adequate subject experience. The dosage information and adjustments are collected and documented for each subject.

***In vitro* animal exposure phase**

First the ex-vivo Frog Palate Exposure Model developed as described above will be used and the performance of all possible *in vitro* assessments. This work can be done in parallel and allow the design of more effective *in vivo* studies. The *in vivo* exposure of mammals will be continued to assess airway clearance and detect potential adverse effects. This approach will help to shorten the development cycle. Airway secretion samples will also be incubated with solutions of different concentrations of the drugs under analysis.

Samples of tissue and airway secretions can be subject to different techniques to assess: cough aerosolization using the simulated cough machine (total mass and size distribution of aerosol) airway clearance

(mucociliary and cough clearance), physical properties (viscoelasticity, surface tension, cohesivity), immunologic analysis, ultra-microscopy, cilia beat frequency in real time using a microscope/video/image acquisition system capable of acquiring digital video images of beating cilia. The main variable to consider in this phase is airway clearance assessment after exposure to different doses of mucomodulators.

Volunteer exposure phase

A group of volunteers (approximately 10) will be asked to participate. Baseline data will include: demographic data, medical history, physical exam, baseline values for critical clinical measurements indicators of response therapy, aerosol post-cough induction pattern, other relevant variables (smoking, alcohol intake, for women date of last menstrual period). The study design for this phase will be a double blind (subject and evaluator), randomized prospective controlled trial. The main variable to consider in this phase will be aerosol pattern while coughing, sneezing and speaking with or without treatment of mucomodulators, as well as to estimate optimum dose and delivery to reduce aerosol formation without compromising mucus clearance.

The methods and compositions of the invention can be used to reduce aerosol formation capability in patients; limit contagiousness of close contacts and decrease the frequency of adverse events. Although, the above-noted studies will be performed on patients with the suspected medical condition, such as SARS, the invention could be applicable to any disease transmitted by droplets or aerosol formed when coughing, sneezing and speaking, such as influenza, TB and SARS.

Example 5 - Respiratory tract mucus clearance.

Mucus simulants (MS) were exposed to airflow in a simulated cough machine (SCM). The MS ranged from non-viscous, non-elastic substances (water - control) to MS of varying degrees of viscosity and elasticity. Mucin crosslinking was increased by adding sodium tetraborate (STB). Mucociliary clearance of the MS was assessed on the frog palate (Figure 5A), elasticity in the *Filancemeter* and the aerosol pattern in a "bulls-eye" target (Figure 6).

The sample loaded was weighed before and after each cough maneuver.

RESULTS:

Mucociliary transport was close to normal speed in viscoelastic samples compared to non-elastic, non-viscous or viscous-only samples. Spinnability ranged from 2.5 ± 0.6 to 50.9 ± 6.9 cm, and the amount of MS expelled from the SCM increased from 47 % to 96 % adding 1.5 µL to 150 µL of STB (Figure 5B). Concurrently, particles were inversely reduced to almost disappear from the aerosolization pattern (Figure 6).

CONCLUSIONS:

The aerosolizability of MS was modified by reducing the number of particles expelled from the SCM, while interfering minimally with its clearance on the frog palate. An unexpected finding is that MS crosslinking increased "expectoration" or cough clearance.

Example 6 – Mucus clearance in anestheitized Dogs

Testing in anesthetized healthy mixed breed dogs (20 - 30 kg), free of respiratory tract infections or other observable respiratory tract infections were administered HMW dextran aerosol (Mucomodulator XL D") (about 500Kda +/- 200 KDa) and a *ca.* 15% increase with borate ("Mucomodulator XL B") (Figure 7).

RESULTS:

Results indicate increased "expectoration" in the cough machine model with normal mucociliary clearance in frog palate testing as well as enhanced tracheal mucociliary clearance in anesthetized dogs, while obtaining a desired target of a significant reduction in fine aerosol formation. An increase in cough clearance using the mucomodulators (traditional mucothickening agents) was especially surprising.

While the present invention has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the invention is not limited to the disclosed examples. To the contrary, the

invention is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

FULL CITATIONS FOR REFERENCES REFERRED TO IN THE SPECIFICATION

1. King M, Brock G, Lundell C. Clearance of mucus by simulated cough. J Appl Physiol 1985; 58: 1776-1782.
2. Jeanneret-Grosjean A, King M, Michoud MC, Lioté H, Amyot R. Sampling technique and rheology of human bronchial mucus. Am Rev Respir Dis 1988; 137: 707-710.
3. Rubin BK, Ramirez O, King M. Mucus-depleted frog palate as a model for the study of mucociliary clearance. J Appl Physiol 1990; 69: 424-429.
4. Tomkiewicz RP, App EM, Zayas JG, Ramirez O, Church N, Boucher RC, Knowles MR, King M. Amiloride inhalation therapy in cystic fibrosis: Influence on ion content, hydration and rheology of sputum. Am Rev Respir Dis 1993; 148: 1002-1007.
5. Hardy JG, Everard M, Coffiner M, Fossion J. Lung deposition of a Nacystelyn metered dose inhaler formulation. J Aerosol Med 1993; 6:37-44.
6. King M, Rubin BK. Rheology of airway mucus: Relationship with clearance function. Chapter 7 of: Takishima T, Shimura S, eds. *Airway Secretion: Physiological Bases for the Control of Mucus Hypersecretion (Lung Biology in Health and Disease Series)* New York: Marcel Dekker, 1994, 283-314.
7. Feng W, Garrett H, Speert DP, King M. Improved clearability of cystic fibrosis sputum with dextran treatment *in vitro*. Am J Respir Crit Care Med 1998; 157: 710-714.
8. King M, Festa E. The evolution of the frog palate model for mucociliary clearance. In: Baum G, ed. *Cilia, Mucus and Mucociliary Interactions.* New York: Marcel Dekker, 1998, 191-201.
9. Finlay WH, Lange CF, King M, Speert DP. Lung delivery of aerosolized dextran. Am J Respir Crit Care Med 2000; 161: 91-97.

TABLE 1

Sputum rheological analysis - reference data

Malcolm King, PhD
Mucobiology Lab, 173 HMRC, Univ of Alberta, Edmonton, AB T6G 2S2

	log G*,1-100 rad/s	MCI (mucociliary clearability index)	CCI (cough clearability index)	G', 10 rad/s (elasticity, dyn/cm2)	η, 10 rad/s (viscosity, Poise)
mean, normal	2.39	0.91	1.52	107 - 490	3.8 - 17.4
(±SD)	0.33	0.08	0.51	(1 SD range)	(1 SD range)
mean, CF	2.80	0.75	0.91	195 - 1780 (1 SD)	7.4 - 70 (1 SD)
(±SD) [Tomk. 1993]	0.48	0.22	0.65	65 - 5750 (2 SD)	2.5 - 200 (2 SD)
mean, CF	3.2	0.7	0.3	740 - 2950 (1 SD)	28 - 110 (1 SD)
(±SD) [App 1998]	0.3	0.1	0.4	370 - 5900 (2 SD)	14 - 225 (2 SD)

tan∂ = mechanical loss tangent = "viscosity/elasticity"

log G*, 1-100 rad/s = mucus rigidity index = vector sum of "viscosity + elasticity"
= mechanical impedance (log scale) averaged over measurement frequency range
(this is the best overall representation of mucus viscoelasticity)

MCI = mucociliary clearability index = 1.62 - 0.22*logG*1 - 0.77*tan∂1
(predicts the normalized clearance rate on frog palate ciliated epithelium)

CCI = cough clearability index = 3.44 - 1.07*logG*100 + 0.89*tan∂100
(predicts the normalized clearance by airflow in simulated cough machine)
(minimum value normally constrained to zero)

G', 10 rad/s = elasticity = shear storage modulus, measured at 10 rad/s (1.7 Hz)

η, 10 rad/s = viscosity = loss modulus/frequency, at 10 rad/s

normal data adapted from Jeanneret-Grosjean et al., Am Rev Respir Dis 1988; 137: 707-710

CF data from meta-analysis of control/baseline sputum from adult patients
(Tomkiewicz et al. Am Rev Respir Dis 1993;148:1002; App et al. Chest 1998;114:171)

10. Vanderbist F, Wery B, Baran D, Van Gansbeke B, Schoutens A, Moes AJ. Deposition of nacystelyn from a dry powder inhaler in healthy volunteers and cystic fibrosis patients. Drug Dev Ind Pharm 2001; 27:205-12.

11. King M, Rubin BK. Pharmacological approaches to discovery and development of new mucolytic agents. Advanced Drug Delivery Reviews 2002; 54: 1475-1490.

12. King M. Mucus, mucociliary clearance and coughing. In: Bates DV. *Respiratory Function in Disease, 3rd ed.* Philadelphia: Saunders, 1989: 69-78.

13. King M. Role of mucus viscoelasticity in cough clearance. Biorheology 1987; 24: 589-597.

14. Zayas JG, Man GCW, King M. Tracheal mucus rheology in patients undergoing diagnostic bronchoscopy: Interrelations with smoking and cancer. Am Rev Respir Dis 1990; 141: 1107-1113.

15. Wanner A, Salathé M, O'Riordan TG. Mucociliary clearance in the airways. Am J Respir Crit Care Med 1996; 154: 1868-1902.

16. King M, Rubin BK. Mucus physiology and pathophysiology: Therapeutic aspects. Chapter 13 of: Derenne JP, Whitelaw WA, Similowski T, eds. *Acute Respiratory Failure in COPD (Lung Biology in Health and Disease Series).* New York: Marcel Dekker, 1996: 391-411.

17. King M, Rubin BK. Mucus controlling agents: Past and present. In: Rau JL, ed. *Aerosolized Drugs for the Respiratory Tract.* Respir Care Clinics N Amer 1999: 575-594.

18. King M, Rubin BK. Pharmacological approaches to discovery and development of new mucolytic agents. Advanced Drug Delivery Reviews 2002; 54: 1475-1490.

What Is Claimed Is:

1. A method of improving respiratory tract mucus clearance in a subject comprising administering an effective amount of a respiratory tract mucothickening agent to said subject.

2. The method of claim 1 wherein the mucothickening agent is selected from the group consisting of those that promote the formation of one or more of the following: covalent bonds, ionic bonds, hydrogen bonds, van der Waals' forces, intermingling, extracellular DNA & F-actin network.

3. The method of claim 2 wherein the mucothickening agent is selected from the group consisting of: high molecular weight dextran, sodium tetraborate, calcium chloride, and a polycationic agent.

4. The method of claim 3, wherein the mucothickening agent is one or more of the said agents.

5. The method of claim 4 wherein the polycationic agent is polylysine.

6. The method of any one of claims 1 – 5 wherein the mucus clearance is mucociliary clearance.

7. The method of any one of claims 1 - 5, wherein the mucus clearance is cough clearance.

8. The method of any one of claims 1 – 7 wherein aerolization of an airborne disease is inhibited in said subject.

ABSTRACT

The invention relates to compositions and methods for improved respiratory tract mucus clearance. The compositions of the invention are mucothickening compositions. They can be used to improve mucus clearance in a subject in need thereof, such as in the treatment of conditions related to non-functioning cilia or to excessively thin mucus. The compositions

Types of Bonds Occurring in a Mucous Gel

1. COVALENT BONDS
- glycoprotein subunits are linked primarily by intramolecular S-S bonds

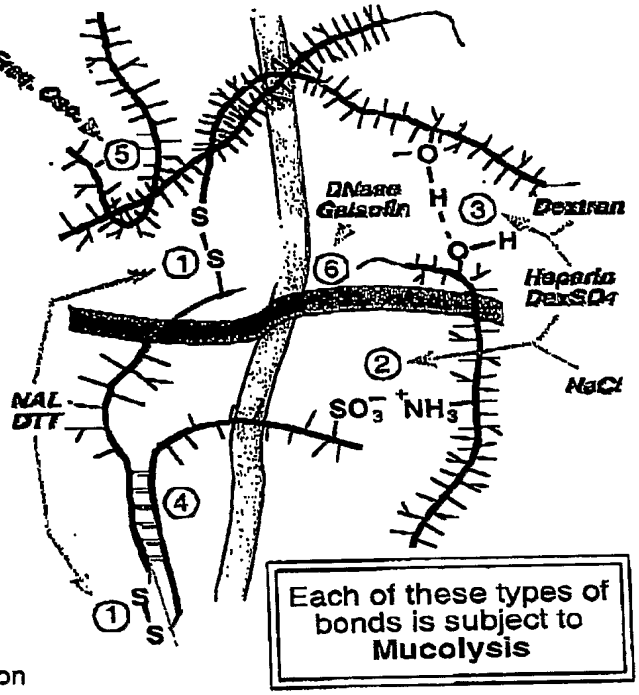
2. IONIC BONDS
- mucin macromolecules have both positive and negative fixed charges, which are capable of interacting

3. HYDROGEN BONDS
- H-bonds link the oligosaccharide side-chains

4. VAN DER WAALS' FORCES
- interdigitation between oligosaccharide moieties may be important

5. INTERMINGLING
- physical entanglements between mucin macromolecules

6. EXTRACELLULAR DNA & F-ACTIN
- parallel network formation in infection



Each of these types of bonds is subject to **Mucolysis**

FIGURE 1

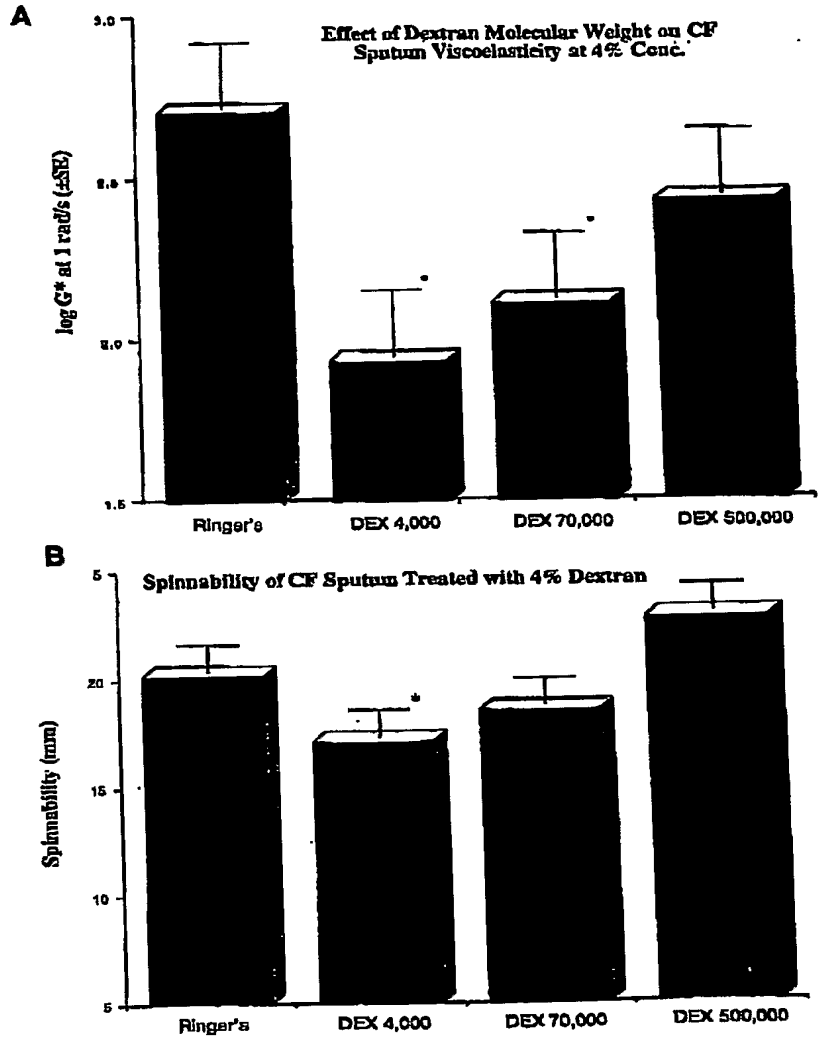
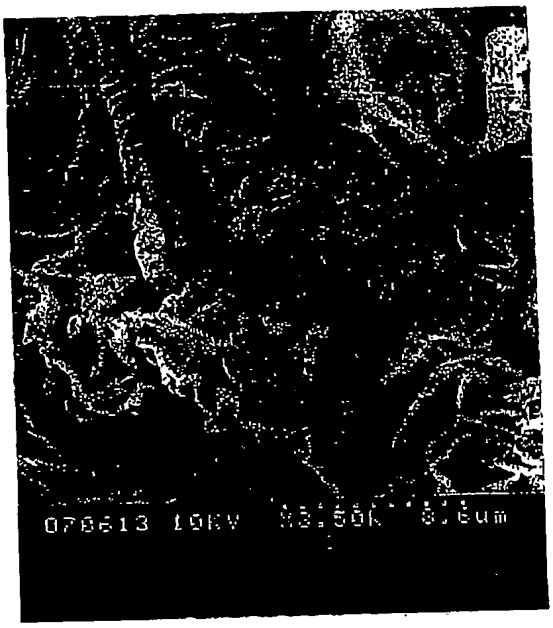


FIGURE 2

A



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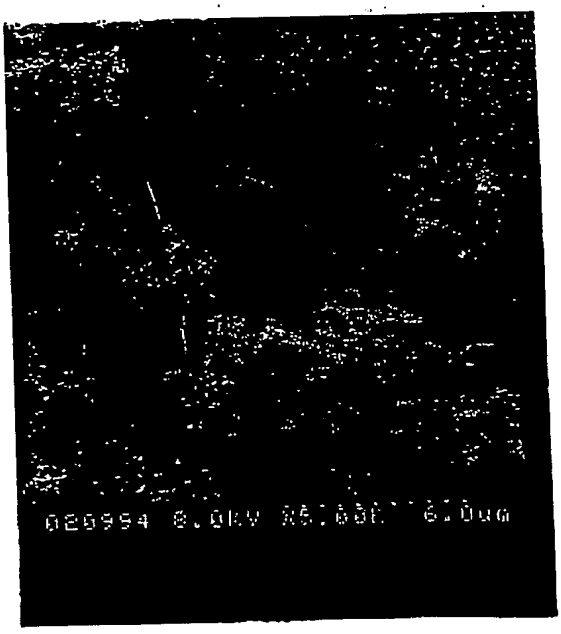


FIGURE 3

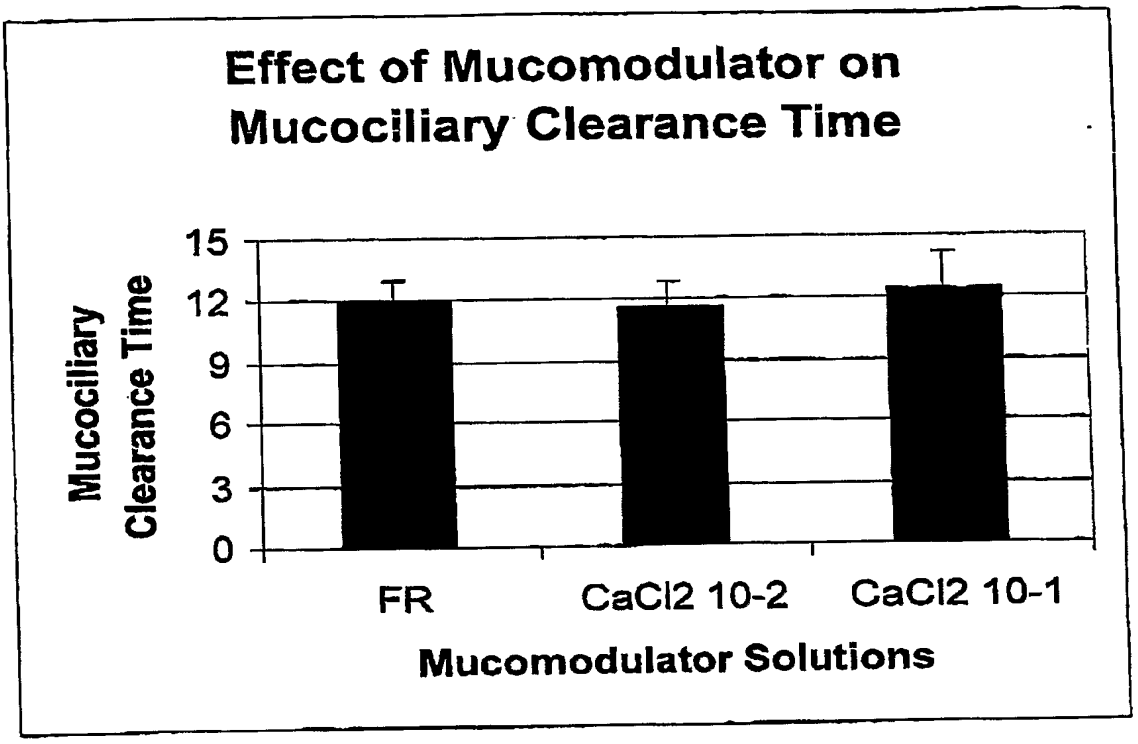
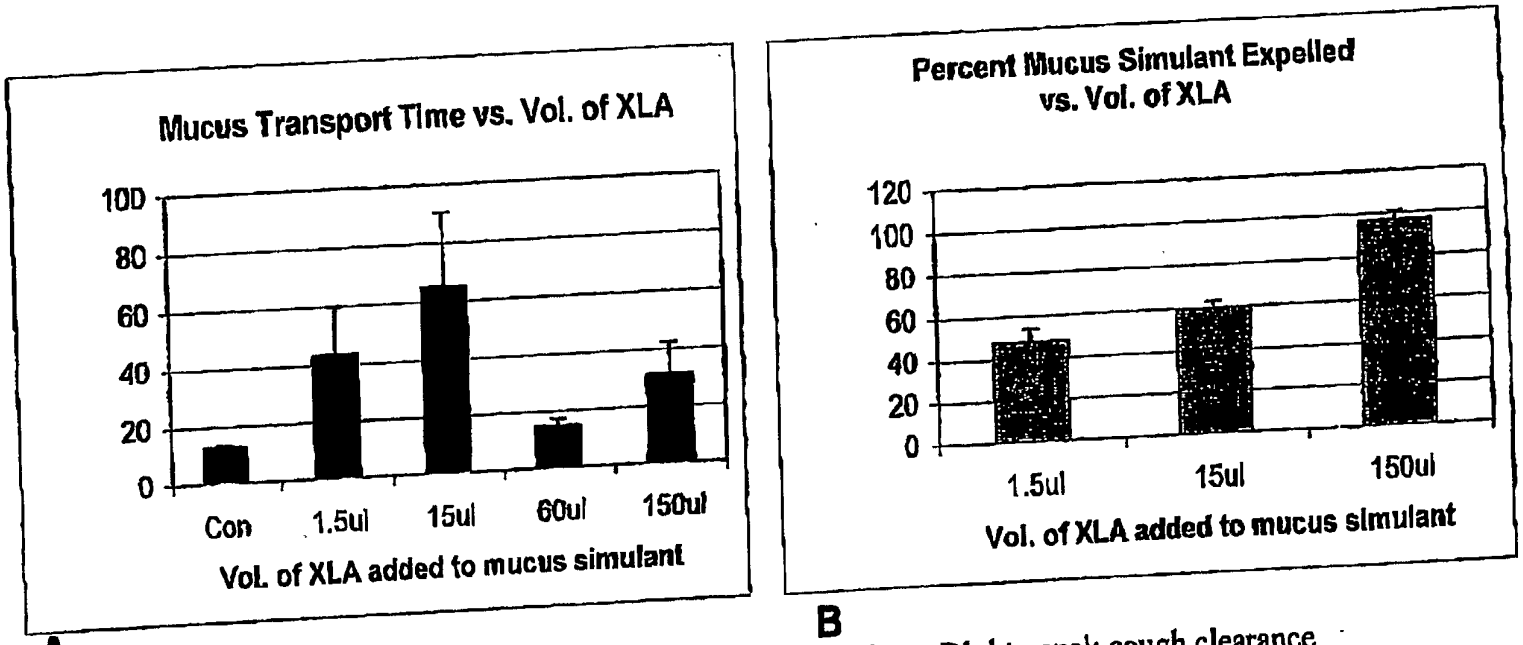


FIGURE 4

Effect of Cross-Linking Agent on Mucociliary Clearance and "Expectoration" from Simulated Cough Machine



Left panel: mucociliary clearance velocity on frog palate epithelium. Right panel: cough clearance ("expectoration", i.e. the percentage of initial load clearing the mouth of the artificial trachea during the cough maneuver. Both the amount of aerosol striking the target and the amount of fine aerosol decreased progressively with added crosslinking agent. Mucociliary clearability matched that of frog mucus control at intermediate STB concentration, while cough clearability continued to increase with increasing STB

FIGURE 5

•Effect of Cross-Linking Agent on the Aerosolization and Dispersion of Synthetic Mucus in a Cough Simulator

Initial experiments with mucomodulation using mucus simulants prepared from locust bean gum (LBG) crosslinked with sodium tetraborate (**STB**) at increasing concentrations (added volumes). Above panels: aerosol patterns at 40 cm. following a standardized cough maneuver.

FIGURE 6

Tracheal Mucociliary Clearance in Anesthetized Dogs Exposed to Saline Solution and Different Concentrations of Mucomodulators: XL"D" and XL"B"

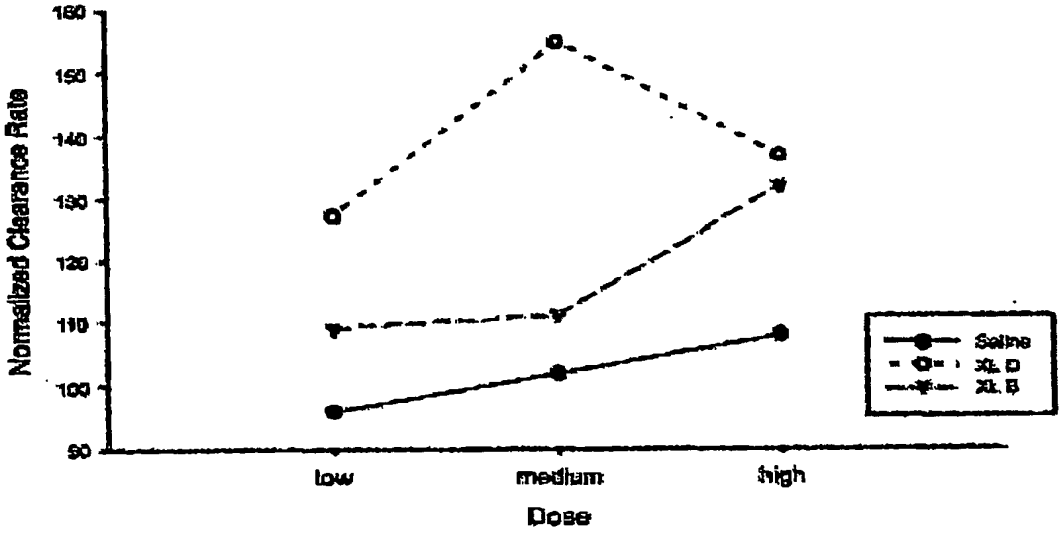


FIGURE 7

Patent Application Data Sheet

Application Information

Application number::	
Application Type::	Provisional
Subject Matter::	Utility
Suggested Classification::	
Suggested Group Art Unit::	
CD-ROM or CD-R?::	None
Number of CD disks::	
Number of copies of CDs::	
Sequence submission?::	
Computer Readable Form (CRF)?::	No
Number of copies of CRF::	
Title::	Compositions And Methods For Improved Respiratory Tract Mucus Clearance
Attorney Docket Number::	11157-76
Request for Early Publication?::	No
Request for Non-Publication?::	No
Suggested Drawing Figure::	
Total Drawing Sheets::	7
Small Entity?::	Yes
Latin Name::	

Variety denomination name::	
Petition included?::	No
Petition Type::	
Licensed US Govt. Agency::	
Contract or Grant Numbers::	
Secrecy Order in Parent Appl.?::	No

Applicant Information

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Representative Information

Representative Customer Number:: **001059**

Domestic Priority Information

Application::	**Continuity Type::**	**Parent Application::**	**Parent Filing Date::**
This Application Or enter the appropriate application serial no.	Continuation of	Enter the parent application number on which priority is claimed No more than 20 characters	MM/DD/YY

Foreign Priority Applications

Country::	**Application Number::**	**Filing Date::**	**Priority Claimed**
			No

Assignee Information

Assignee name::
Street of mailing address::
City of mailing address::
State or Province of mailing address::
Country of mailing address::

Postal or Zip Code of
mailing address::